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Application Number	Filed
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US005851832A

United States Patent [19]

Weiss et al.

[11] Patent Number: 5,851,832

[45] Date of Patent: Dec. 22, 1998

[54] **IN VITRO GROWTH AND PROLIFERATION OF MULTIPOTENT NEURAL STEM CELLS AND THEIR PROGENY**

[75] Inventors: **Samuel Weiss**; **Brent Reynolds**, both of Alberta, Canada; **Joseph P. Hammang**; **E. Edward Baetge**, both of Barrington, R.I.

[73] Assignee: **Neurospheres, Ltd.**, Canada

[21] Appl. No.: **486,648**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 270,412, Jul. 5, 1994, abandoned, which is a continuation of Ser. No. 726,812, Jul. 8, 1991, abandoned, and a continuation-in-part of Ser. No. 385,404, Feb. 7, 1995, abandoned, which is a continuation of Ser. No. 961,813, Oct. 16, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 359,945 Dec. 20, 1994, abandoned, which is a continuation of Ser. No. 221,655, Apr. 1, 1994, abandoned, which is a continuation of Ser. No. 967,622, Oct. 28, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, and Ser. No. 376,062, Jan. 20, 1995, abandoned, which is a continuation of Ser. No. 10,829, Jan. 29, 1993, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 149,508, Nov. 9, 1993, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 311,099, Sep. 23, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 338,730, Nov. 14, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812.

[51] **Int. Cl.**[6] **C12N 5/06**; C12N 5/08; C12N 5/02

[52] **U.S. Cl.** **435/368**; 435/325; 435/366; 435/383; 435/384

[58] **Field of Search** 435/240.2, 325, 435/366, 368, 377, 383, 384

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,753,635	6/1988	Sagen et al.	604/49
4,980,174	12/1990	Sagen et al.	424/563
5,082,670	1/1992	Gage	424/520
5,175,103	12/1992	Lee et al.	435/172.3
5,411,883	5/1995	Boss et al.	435/29
5,612,211	3/1997	Wilson et al.	435/378

FOREIGN PATENT DOCUMENTS

0 233 838	8/1987	European Pat. Off. .
89/03872	5/1989	WIPO .
90/06757	6/1990	WIPO .
91/02003	2/1991	WIPO .
91/09936	7/1991	WIPO .
91/17242	11/1991	WIPO .
93/01275	1/1993	WIPO .
93/09802	5/1993	WIPO .
94/03199	2/1994	WIPO .

OTHER PUBLICATIONS

Almazan et al., "Epidermal Growth and Bovine Growth Hormone Stimulate Differentiation and Myelination of Brain Cell Aggregates in Culture," *Developmental Brain Research,* 21:257–264 (1985).

Anchan et al., "Trophic Factors Influence Proliferation of Germinal Neuroepithelial Cells of the Retina," *J. Cell Biol.,* 109:58a, Abstract No. 308 (1989).

Anchan et al., "EGF and TGF–α Stimulate Retinal Neuroepithelial Cell Proliferation in Vitro," *Neuron,* 6(6):923–936 (1991).

Bayer et al., "Neuron production in the Hippocampus and olfactory bulb of the adult rat Brain: addition or replacement?", *Annals NY. Acad. Sci.* 457:163–172 (1985).

Björklund et al., "Neural Grafting in Animal Models of Neurodegenerative Diseases," *Ann. New York Acad. Sci.,* 457:53–81 (1985).

Bouvier et al., "Basic Fibroblast Growth Factor (bFGF) Promotes the Survival and Proliferation of Mesencephalic Neuronal Precursors in Vitro," *Society for Neuroscience Abstracts,* vol. 18, Abstract No.: 403.7 (1992).

Boyles et al., "Accumulation of Apolipoproteins in the Regenerating and Remyelinating Mammalian Peripheral Nerve," *J. Biol. Chem.,* 265(29):17805–17815 (1990).

Calof et al., "Analysis of Neurogenesis in a Mammalian Neuroepithelium: Proliferation and Differentiation of an Olfactory Neuron Precursor in Vitro," *Neuron,* 3:115–127 (1989).

Cattaneo et al., "Identifying and Manipulating neuronal stem cells," *TINS,* 14(8): 338–340 (1991).

Cattaneo et al., "Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor," *Nature,* 347:762–765 (1990).

Cepko "Immortalization of neural cells via retrovirus–mediated oncogene transduction," *Ann. Rev. Neurosci.,* 12:47–65 (1989).

Deloulme et al., "Establishment of Pure Neuronal Cultures From Fetal Rat Spinal Cord and Proliferation of the Neuronal Precursor Cells in the Presence of Fibroblast Growth Factor," *Journal of Neuroscience Research,* 29:499–509 (1991).

Dunnett et al., "Dopamine–rich transplants in experimental Parkinsonism," *TINS,* 266–270 (Jul. 1983).

Emerich et al., "Behavioral Effects of Neural Transplantation,"*Cell Transplantation,* 1:1–27 (1992).

Faaland et al., "Rapid uptake of tyrphostin into A431 human epidermoid cells is followed by delayed inhibition of epidermal growth factor (EGF)–stimulated EGF receptor tyrosine kinase activity", *Mol. Cell Biol.* 11(5):2697–2703 (1991).

(List continued on next page.)

Primary Examiner—George C. Elliott
Assistant Examiner—Johnny F. Railey, II
Attorney, Agent, or Firm—Flehr Hohbach Test Albrition & Herbert LLP

[57] **ABSTRACT**

A method for the in vitro proliferation and differentiation of neural stem cells and stem cell progeny comprising the steps of (a) isolating the cells from a mammal, (b) exposing the cells to a culture medium containing a growth factor, (c) inducing the cells to proliferate, and (d) inducing the cells to differentiate is provided.

80 Claims, 3 Drawing Sheets